CHANGES IN THE MUTAGENIC ACTION OF HYDROXYLAMINE ON T-2 PHAGE

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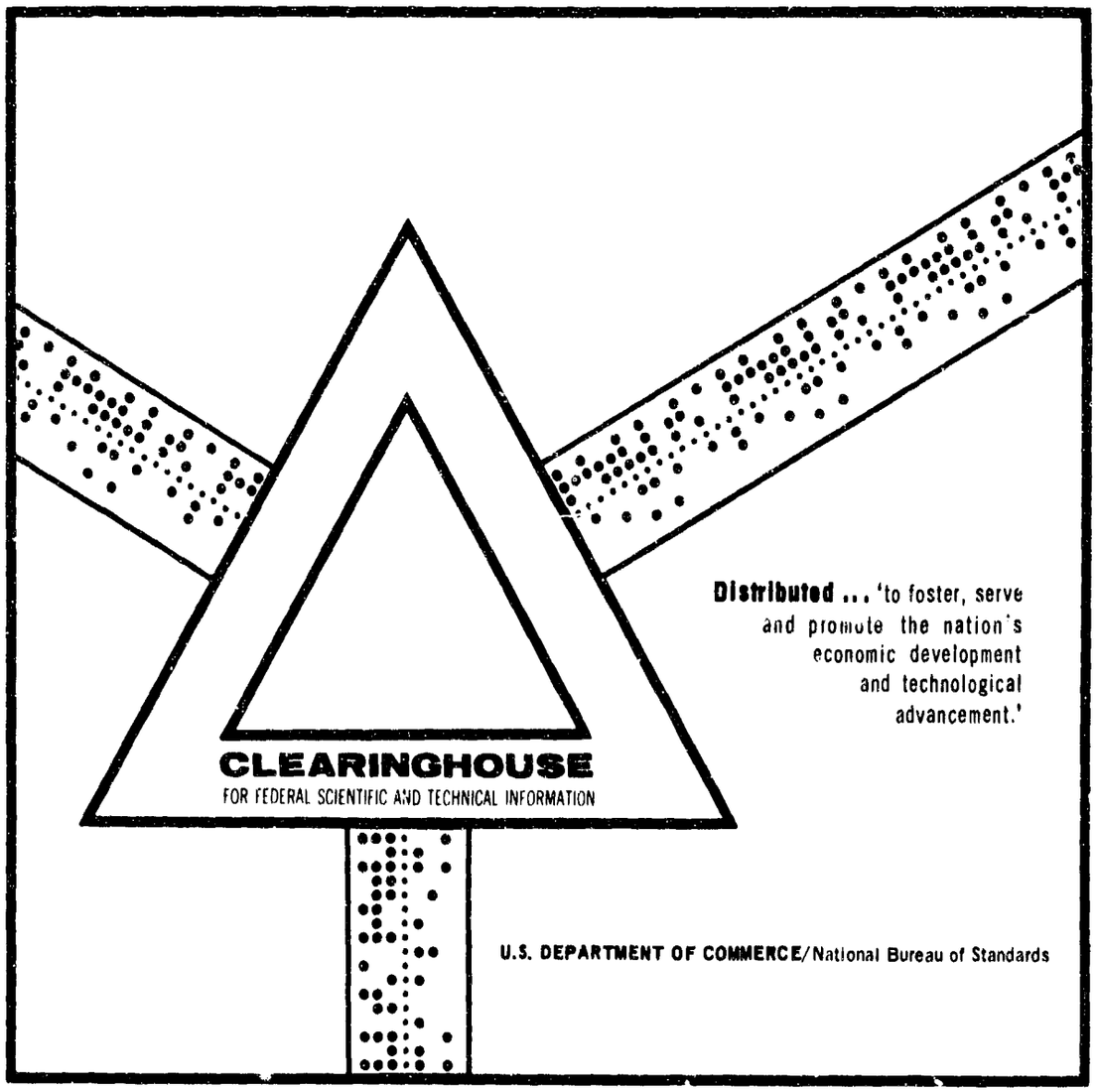

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[Following is the translation of an article by V. F. Chubuchkov, Institute of Epidemiology and Microbiology imeni N. F. Gamaleya, AMN SSSR, published in the Russian-language periodical Zhurnal Mikrobiologii, Epidemiologii, i Immunobiologii (Journal of Microbiology, Epidemiology, and Immunobiology) No 6, 1965, pages 129-33. It was submitted on 5 Jun 1964.]

It is known that the chemical activity of cytosine (or some of its derivatives) in DNA during the reaction with hydroxylamine differs from the activity of this base in free nucleotide (Brown and Schell, 1961; Freese and associates, 1961; and others). This difference may be conditioned by two causes: first, the DNA is a negatively charged polymer, and secondly, in two-thread DNA the bases are sterically fixed by means of the formation of hydrogen bonds between the pairs of bases in the two complementary chains of DNA. Therefore any specific cytosine base will react with hydroxylamine only upon disruption of the entirety of the hydrogen bonds. It is necessary to note that in the scheme of the reaction of hydroxylamine with cytosine (Verwoerd and associates, 1961) there is a joining of the second molecule of hydroxylamine with the liberation of ammonia. The ease with which this reaction flows should be determined by the lability of the amino group of cytosine at the 6th carbon atom, which by means of hydrogen is bound with guanine.

It is known that heating leads to breakdown of the hydrogen bonds between the two chains of DNA. Thus at a temperature close to 83° there is a complete splitting of threads in the DNA of T-2 bacteriophage. Consequently the amount of cytosine in the active state should increase with an increase of temperature. In actuality in thymus DNA a considerable increase was noted in the rate of the reaction of hydroxylamine with cytosine in comparison with the analogous reaction in native DNA. By itself hydroxylamine did not exert any noticeable denaturing effect on DNA, and also did not influence its hyperchromic effect (Morozova and Salganik, 1964). At the same time the matrix activity of DNA is reduced more sharply after treatment with hydroxylamine of denatured and not native DNA (Belman, 1964). It is interesting to note that only treatment of denatured (heated) transforming DNA (Hemophilus) with nitrous acid made it possible to obtain a large number of transformed resistant markers to 5 various antibiotics (Horn and Merriott, 1962).

In the work by Freese and Strack (1962) it was shown that treatment of transforming DNA of the prototrophic form of B. subtilis with hydroxylamine at a temperature of 60-90° or under other conditions which disrupt the completeness of the hydrogen bonds (high concentration of NaCl in the solution, glycol) is accompanied by considerable intensification of the mutagenic activity of hydroxylamine. It is probable that the effect of intensification should be different in the induction of mutations for various organisms or in some specific organism on diverse genetic loci, if one keeps in mind the possibility of their different partial "protective ability" from mutagen (Loveless, 1960). The different lability of loci may be determined both by their various extent on the genetic map and by their heterogeneity on pairs of nitrogen bases.

An analysis, conducted by Freese and Strack, shows that native DNA isolated from microbes possesses a lesser capacity to react with mutagen than DNA which is packed in the head of a phage particle (frequency of mutation in T-4 phage was greater by approximately 1000 times than in transforming DNA of B. subtilis). This means that specific structures in phage DNA are more accessible for mutagen than in DNA of B. subtilis.

During an investigation of the reaction of hydroxylamine with cytosine in RNA and in DNA it was noted (Schuster, 1961) that for the same time of incubation the change of cytosine in RNA was more significant (RNA is partially single-chain). However, also in DNA specific sectors of the molecule are denatured even at 25° and intramolecular denaturation increases with an increase of temperature (Beer and Thomas, 1961; Geiduschek, 1962; Freifelder and Davison, 1963).

Stemming from what was said it can be expected that treatment of extracellular bacteriophage T-2 with hydroxylamine at increased temperature should lead to an increase in the rate of the reaction with hydroxymethylcytosine in phage DNA both in the initial and intermediate phases and cause a considerable mutagenic effect. At the same time it was also possible to expect that the increase in the rate of mutation would be different on separate genetic loci. We decided to check the correctness of both proposals. With this goal an analysis was made of induction of r- and h^c-mutations following treatment of extracellular T-2 phage with 1 M solution of hydroxylamine (+1 M NaCl at 60°, pH 6.0). The action of mutagen was terminated by means of dilution of treated phage in Difco broth. As indicator bacterial strains we used E. coli B - during analysis of total amount of active phage particles after treatment with mutagen, analysis of induction of r-mutations, and for preadsorption of treated phage and E. coli 32 - as the indicator line during analysis of induction of h^c-mutations.

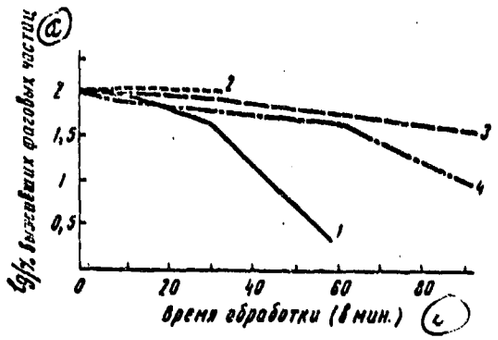

Figure 1. Treatment of extracellular T-2 phage with 1 M solution of hydroxylamine (+1 M NaCl), pH 6.0, 60°, and also endurance in acetate buffer (pH 3.0, 1 M NaCl, 37°) of phage after short-term treatment with hydroxylamine.
1 - Treatment with hydroxylamine (pH 6.0, 60°); 2 - endurance at 60° of untreated phage (control); 3 - endurance of treated phage in acetate buffer; 4 - endurance of untreated phage in acetate buffer (control).
Key: (a) lg% of surviving phage particles; (b) time of treatment (in minutes).

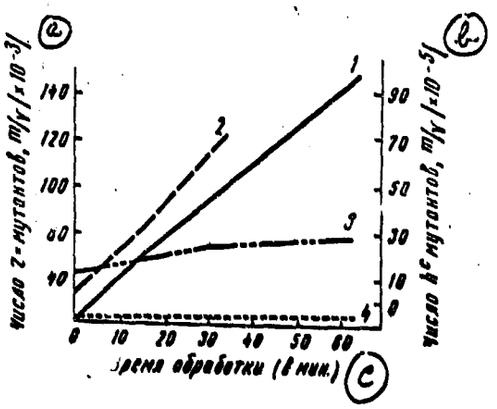

Figure 2. Induction of r- and h^0-mutations of T-2 phage during treatment with hydroxylamine (pH 6.0, 60°) and when treated phage is kept in acetate buffer (number of mutants to surviving phage particles).
1 - Induction of r-mutations; 2 - induction of h^0-mutations; 3 - induction of r-mutations during maintenance of treated phage in acetate buffer; 4 - tests on r-mutants during maintenance of untreated phage in acetate buffer (control).
Key: (a) number of r-mutants, m/V/x10-5/; (b) number of h^0-mutants, m/V/x10-5/; (c) time of treatment (in minutes).

The death curve for T-2 phage (Figure 1) during treatment with hydroxylamine (60°) was multishock. However, death of phage set in only after a certain interval of time. The number of h^c-mutants in respect to surviving increased linearly throughout the entire period of treatment, while the induction of r-mutations for this same interval of time followed a more complex dependence (Fig. 2).

During the calculation of mutagenic activity of hydroxylamine it can be assumed without great error that during the first 10 minutes of treatment the rate of survival comprised 100% and, consequently, induction - the increase of absolute number of mutant forms for this interval of time - can be expressed as a function of dose of mutagen - time of treatment. The time of treatment, equal to 60 minutes, made up the conditional unit of dose.

Induction of both r- and h^c-mutations at 60° proceeded at a considerably greater rate than at 37° (Figure 3).

Another peculiarity of the process of mutation of phage at 60° was the considerably greater lability of the r-system in comparison with the h-system.

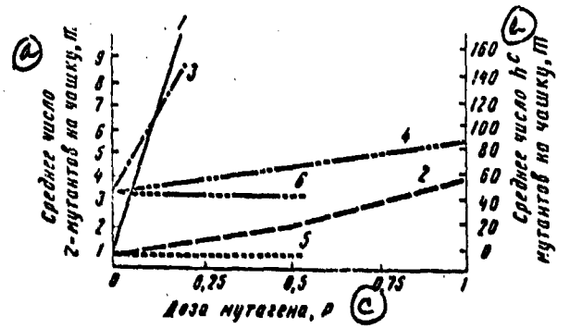

Figure 3. Induction of r- and h^c-mutations of T-2 phage during treatment with hydroxylamine (pH 6.0, 60°) (absolute values). 1 - induction of r-mutations (60°); 2 - induction of r-mutations (37°) (control); 3 - induction of h^c-mutations (60°); 4 - induction of h^c-mutations (37°) (control); 5 - tests on r-mutants (untreated phage) (control); 6 - tests on h^c-mutants (untreated phage) (control).
Key: (a) Average number of r-mutants per dish, m; (b) Average number of h^c-mutants per dish, m; (c) Dose of mutagen, D.

On the basis of recombination genetic tests it is proposed that at the time when r segments on the genetic map of T-2 bacteriophage (rI, rII, rIII) have a quite large extent, the h-locus is considerably smaller in size (Shtrayzinger and Franklin, 1956). Since the action of hydroxylamine on T-2 phage DNA is accompanied only by a change in hydroxymethylcytosine, then the difference in the sensitivity of the two genetic areas, which was noted during treatment of mutagen at 60°, may be connected with their various length or with heterogeneity on pairs of bases of guanine-hydroxymethylcytosine (G-HMC). Sectors of DNA with accumulations of pairs of G-HMC are more stably bound and, consequently, should be more resistant to heating. Heating up to 60° alone did not lead to an increase of mutant forms. Therefore mutagenesis under these conditions cannot be explained, taking into consideration only the possibilities of a certain depurinization of DNA (Freese, 1959).

It was shown in a number of works that the mutagenic activity of hydroxylamine can be changed if extracellular T-4 bacteriophage is treated (Schuster and Vielmetter, 1961), and also the RNA of tobacco mosaic virus (Schuster and Wittman, 1963) with various pH values for the medium. An increase in the induction of mutants was also observed with an increase in the concentration of hydroxylamine (Freese et al., 1961; Freese and Strack, 1962).

According to the arrangement of Verwoerd, following treatment of cytosine with hydroxylamine several intermediate reaction products are formed. However, up to now it is unknown which of these phases is the most responsible for the mutation effect, since the rates of the reactions in each of the phases have been studied little. Based on certain findings (Schuster and Wittman, 1963, Freese, 1961), both the intermediate reaction products and also the end compound (uracil) may take up the function of thymine, thus ensuring the possibility of localized mutation. Here the initial pair of G-HMC bases convert into an A-T pair. The converted cytosine will act as an analog of thymine due to the fact that, first of all, an atom of hydrogen at N-1 can form one bond with the N-1 of adenine (but does not interact sterically with the hydrogen atom N-1 of guanine), and, secondly, it is namely the hydroxyl amino- or keto-group at the 6th carbon atom of changed cytosine which can serve as the H-acceptor, promoting the formation of a bond with the amino-group of adenine. It can be assumed that the basic mutation effect of hydroxylamine is connected with the formation of uracil or thymine-like compounds, since the transition of other intermediate reaction products into rare tautomeric, thymine-like forms should be connected with a supplementary expenditure of energy for their formation.

The goal of this work was a check of the correctness of this proposal. We studied the induction of r-mutations in extracellular T-2 phage which was treated with hydroxylamine (0.7°) for a brief

interval of time (5 hours). Then we traced the endurance of phage for several hours in an acetate buffer (pH 3.0, 37°). Based on the arrangement of the reaction of hydroxylamine with cytosine at a low pH value for the medium there is a greater formation of uracil. In that interval of time we took probes for an analysis of the number of active phage particles and mutants. Removal of mutagen was achieved by 18-hour dialysis in running water. The control was phage, which during the same interval of time was found in an acetate buffer (see Fig. 3).

The results of the tests showed (Fig. 3) that survival of hydroxylamine-treated phage (at pH 3.0) was accompanied by an increase in the number of r-mutants. At the same time in a control experiment only inactivation of phage was noted, and not the induction of r-mutations.

Conclusions

1. Treatment of extracellular T-2 bacteriophage with hydroxylamine at 60° led to an increase in the rate of induction of r- and h-mutants in connection with an increase in the rate of the reaction of hydroxylamine with hydroxymethylcytosine in phage DNA due to a break in the hydrogen bonds and an increase in the initial rate of the reaction.

2. The different increase in the rate of mutation in r- and h-genetic loci was conditioned by the heterogeneity of these areas based on G-HMC pairs of bases. By means of the selection of temperature in combination with treatment by mutagen, it is probably possible to achieve a greater selectivity of mutation.

3. The change in the course of the reaction of hydroxylamine with cytosine (hydroxymethylcytosine in DNA of T-2 phage) to the side of formation of uracil or thymine-like compounds, which to a greater degree than other intermediate compounds may be responsible for mutation changes, led to an increase in the rate of induction of r-mutations.

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